CLAIMS

1. A method for RNA or polypeptide synthesis from a DNA template comprising the steps of

   a) providing a cell-free system enabling RNA or polypeptide synthesis from a DNA template, said DNA template comprising a promoter with at least one UP element;

   b) performing said RNA or polypeptide synthesis;

   c) recovering said synthesized RNA or polypeptide;

   characterized in that the concentration of α subunit of RNA polymerase, but not of other subunits, is increased in said cell-free system, comparing to its natural concentration existing in the cell-free system.

2. The method according to Claim 1, wherein said system enabling RNA or polypeptide synthesis from a DNA template is a cell-free system comprising a bacterial cell-free extract.

3. The method according to Claim2, wherein the promoter on the DNA template includes sequence from the *argC* gene promoter of *Bacillus stearothermophilus*, preferably, the sequence from nucleotide – 89 to +1 when the latter is the first nucleotide in mRNA of the *argC* gene.

4. The method according to Claim 2 or 3, wherein said cell-free system further comprises purified thermostable RNA polymerase holoenzyme.

5. The method according to Claim 4, wherein said thermostable RNA polymerase holoenzyme is from *Thermus thermophilus*.

6. The method according to any of Claims 2 to 5, wherein the concentration of α subunit of RNA polymerase is increased by adding purified α subunit of RNA polymerase to the bacterial cell-free extract.

7. The method according to Claim 6, wherein said purified α subunit is added to a final concentration comprised between 15 µg/ml and 200 µg/ml.

8. The method according to Claim 6 or 7, wherein the cell-free extracts is prepared from cells overexpressing a gene encoding α subunit of RNA polymerase.

9. A method for the production of a protein from a DNA template in a cell-free system characterized in that it comprises the steps of

a) providing in a reaction mixture, a bacterial cell-free system enabling the coupling of *in vitro* transcription of a specific gene from a DNA template, and the corresponding protein synthesis ;

b) adding to the reaction mixture the DNA template encoding the desired protein and purified α subunit of the RNA-polymerase; and,

c) optionally, adding a thermostable RNA polymerase,

d) recovering the produced protein.

10. The method according to Claim 9, wherein said added thermostable RNA polymerase is from *T. thermophilus*.

11. The method according to Claim 9 or 10, wherein said purified α subunit is added to a final concentration comprised between 15 µg/ml and 200 µg/ml.

12. The method according to any of Claims 9 to 11, wherein a DNA-binding regulatory protein is further added to the reaction mixture at step (b).

13. The method according to any of Claims 9 to 12, wherein said DNA template comprises an amplification product of an Open Reading Frame encoding the desired protein.

14. The method according to Claim13, wherein said DNA template further comprises an additional DNA fragment, which is at least 3 bp long, preferably longer than 100 bp and more preferably longer than 200 bp, located immediately downstream the stop codon of said Open Reading Frame.

15. The method according to Claim 13, wherein said DNA template further comprises an additional DNA fragment containing a transcriptional terminator.

16. The method according to Claim 13, wherein said transcriptional terminator is the T7 phage transcriptional terminator.

17. A reaction mixture for cell-free protein synthesis characterized in that it is prepared from cells which overexpress the gene encoding α subunit of the RNA polymerase.

18. A reaction mixture for cell-free protein synthesis characterized in that it comprises a bacterial cell-free extract and an amount of purified α subunit of RNA polymerase.

19. The reaction mixture of Claim 17 or 18, wherein said purified or overexpressed α subunit of RNA polymerase is at a concentration comprised between 15 µg/ml and 200 µg/ml in the reaction mixture.

20. The reaction mixture of any of Claims 17 to 19 , characterized in that it further comprises a DNA template comprising a gene encoding a protein of interest under the control of a promoter with at least one UP element.

21. The reaction mixture according to Claim 17, characterized in that it further comprises a DNA-binding regulatory protein.

22. A kit for cell-free RNA and/or protein synthesis characterized in that it comprises the following components:

a) a cell-free extract, preferably *E. coli* S30 cell-free extract;

b) purified α subunit of RNA polymerase;

c) optionally, appropriate buffers and compounds for carrying out *in vitro* transcription and/or translation reaction;

d) optionally, amino acid mixture lacking one amino acid.

23. A kit for cell-free RNA and/or protein synthesis characterized in that it comprises the following components:

a) a cell-free extract, preferably *E. coli* S30 cell-free extract, wherein said cell-free extract is obtained from cells overexpressing subunit of RNA polymerase;

b) optionally, appropriate buffers and compounds for carrying out *in vitro* transcription and/or translation reaction;

c) optionally, amino acid mixture lacking one amino acid.

24. The kit according to Claim 22 or 23, wherein said purified or overexpressed α subunit is from *E. coli* strains.

25. Use of a purified α subunit of RNA polymerase for enhancing protein synthesis in a cell-free system, wherein said cell-free system comprises a DNA template with at least one UP element.

26. The use according to Claim 25, wherein said purified α subunit of RNA polymerase is added in a cell-free system at a concentration comprised between 15 µg/ml and 200 µg/ml.

27. The use according to Claim 26, wherein said purified α subunit of RNA polymerase is added in a cell-free system together with a thermostable RNA polymerase holoenzyme, preferably of *T. thermophilus*.

ANNEX 1: Sequence of the *ompF* gene promoter used in the experiment described in D1

gatcatcctg ttacggaata ttacattgca acatttacgc gcaaaaacta atccgcattc

ttattgcgga ttagtttttt cttagctaat agcacaattt tcatactatt ttttggcatt

ctggatgtct gaaagaagat tttgtgccag gtcgataaag tttccatcag aaacaaaatt

tccgtttagt taatttaaat ataaggaaat catataaata gattaaaatt gctgtaaata

tcatcacgtc tctatggaaa tatgacggtg ttcacaaagt tccttaaatt ttacttttgg

ttacatattt tttctttttg aaaccaaatc tttatctttg tagcactttc acggtagcga

-35

aacgttagtt tgaatggaaa gatgcctgca gacacataaa gacaccaaac tctcatcaat

-10

agttccgtaa atttttattg acagaactta ttgacggcag tggcaggtgt cataaaaaaa

accatgaggg taataaataa tg